THE ROYAL SOCIETY

doi 10.1098/rstb.2001.0871

Simian immunodeficiency virus in kidney cell cultures from highly infected rhesus macaques (*Macaca mulatta*)

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Trace amounts of simian immunodeficiency virus (SIV) proviral DNA were detected in monolayers of primary kidney cells from two rhesus macaques (*Macaca mulatta*) heavily infected with the highly pathogenic strain SIVmac251. There was no detectable infectious SIV in the supernatant from the kidney cell cultures obtained from either monkey. However, infectious SIV was rescued by co-culture of kidney cells with a permissive lymphoid cell line. Macrophages, present in these cultures, may be the reservoir for the proviral genomes.

Keywords: macaque; kidney; primary cells; simian immunodeficiency virus; macrophage

1. INTRODUCTION

Monolayer cultures of simian kidney cells are an important tool in virological research and have been used for producing therapeutic materials including viral vaccines. The presence of adventitious agents derived from the donor animal could compromise the usefulness of such cultures. Previous investigations centred around the presence of potentially oncogenic viruses such as simian virus 40 or adenoviruses. The discovery of simian immunodeficiency viruses (SIV) in a considerable proportion of several non-human species raises new issues. The capacity of primary kidney cell cultures (PKCCs) to produce SIV has not been extensively investigated, but the involvement of kidney dysfunctions in disease caused by SIV, human immunodeficiency viruses (HIVs) or chimeric constructs (SHIV) (Stephens *et al*. 1998; Humphreys 1995; Liu *et al*. 1999) suggests viral presence and activity in kidney tissues in the infected host. In this report, we describe the characteristics of SIV detected in PKCCs from experimentally infected rhesus macaques. SIV DNA genomes can persist, unexpressed, in the primary cell culture for a period of at least 12 days; however, we were able to rescue a fully infectious virus from PKCCs from one of two animals by co-culture, followed by prolonged passage in a highly susceptible indicator lymphoid cell line.

2. MATERIAL AND METHODS

Rhesus macaques (*Macaca mulatta*), Mmu 28824 and Mmu 29697, are colony-bred animals free of simian type-D retrovirus, SIV and simian T-lymphotropic virus; these animals were housed at the California Regional Primate Research Center (CRPRC) at Davis, CA, in accordance with American Association for Accreditation of Laboratory Animal Care Standards. All procedures were conducted according to the *Guide for the care and use of laboratory animals* (National Research Council 1996). Each monkey was inoculated in the oral cavity twice the same day with 1 ml doses of uncloned, pathogenic SIVmac251. The challenge virus was prepared by sequential passage in rhesus macaques followed by *in vitro* amplification in peripheral blood mononuclear cells (PBMCs) from the same species. The *in vitro* titre of that batch was $10^5\,ml^{-1}$ of 50% tissue culture infective doses ($TCID_{50}$). Plasma, spleen and kidney samples were collected at necropsy, four months after infection. Fragments (10–20 mg) of spleen and kidney tissue collected at necropsy were snap frozen in ethanol on dry-ice, then stored at 80 °C.

DNA was prepared by digestion of tissue fragments by proteinase K ($0.5\,mg\,ml^{-1}$) in 50 mM Tris buffer pH 7.5 containing 25 mM ethylenediaminetetraacetic acid, 1% sodium dodecyl sulphate and 300 mM NaCl, followed by three cycles of extraction into phenol–chloroform and precipitation by isopropanol. DNA purity was estimated by spectroscopy at 260 and 280 nm on pellets redissolved in 200 µl of pure water. A ratio of 1.7 indicated minimal contamination of all DNA samples. Viral RNA was prepared from plasma samples (140 µl) using the QIAmp[1] Viral RNA Mini Kit (ref. 52906; Qiagen, Valencia, CA, USA) following the manufacturer's recommendations.

Kidney cells were prepared by mincing fresh tissue fragments in cooled serum-free Roswell Park Memorial Institute (RPMI) 1640 medium, then seeding T25 or T75 tissue culture flasks or plastic Lab-Tek[1] slides (ref. 177410; Nalge Nare International, Naperville, IL, USA) at $6 \times 10^4\,cells\,ml^{-1}$ in Dulbecco's modified Eagle's minimum essential medium supplemented with 10% foetal calf serum to obtain PKCCs. Half the medium was removed and replaced by fresh medium every three days, and the PKCCs attained confluence after 10–12 days. Most cells exhibited epithelial or fibroblastic morphology. DNA was

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Table 1. *Clinical markers, quantification of SIV genomes in plasma (RNA) and in kidney, spleen and PKCC monolayers (DNA) and detection of p27 GAG antigen in PKCC fluid*

Mmu	clinical signs	CD4 per µl of blood	RNA copies per ml of plasma	DNA copies per µg of DNA			CEMx174 co-culture	p27 capture assay
				spleen	kidney	PKCC monolayer		
28824	liquid stools, weight loss	894	122×10^6	6445	571	67	CPE, p27+ passage 6	**negative** days 6, 12
29697	no sign	491	8×10^6	3565	86	28	negative	**negative** days 6, 12

extracted from the cultured cells, after trypsinization and washing, by resuspension in DNAzol[1] (ref. DN127; Molecular Research Center, Inc., Cincinnati, OH, USA) at 10^7 cells ml^{-1}.

3. RESULTS

(a) *Infection of macaques with pathogenic SIV*

The two young adult male rhesus macaques, Mmu 28824 and Mmu 29697, respectively four and three years old, were infected with the highly pathogenic strain SIVmac251. To maintain a high degree of virulence, the challenge virus was prepared by sequential passage in rhesus macaques followed by *in vitro* amplification in PBMCs from the same species. The *in vitro* titre of that batch was over 10^5 $TCID_{50}$ ml^{-1}. Each monkey was inoculated in the oral cavity twice the same day with 1 ml doses of virus. These monkeys were killed four months after infection. Both macaques still had $CD4^+$ T-cell counts within the normal range for young adult rhesus macaques. Mmu 28824 was observed to have occasional liquid stool and weight loss, but maintained a $CD4^+$ T-cell count of 894 μl^{-1} blood at the time of necropsy. Mmu 29697 had no clinical symptoms and a $CD4^+$ T-cell count of 491 μl^{-1} blood (table 1).

(b) *SIV in infected macaques*

Viral load was estimated in plasma samples from the donor monkeys using the TaqMan[1] assay (Applied Biosystems, Foster City, CA, USA) based on real-time polymerase chain reaction (PCR) after reverse transcription of the viral RNA. Proviral copy number in 100 ng samples of total DNA from tissue samples and PKCCs was determined by the same assay without reverse transcription. As shown in table 1, monkey Mmu 28824 had greater amounts of viral RNA in its plasma, and correspondingly higher quantities of proviral DNA in all cell samples, than did monkey Mmu 29697. PKCC produced low positive results for proviral DNA, but no viral p27 was detected in the culture supernatants by immunocapture assay (SIV Core Antigen, Beckman Coulter Inc., Fullerton, CA, USA), suggesting strong restriction of viral proliferation.

To evaluate whether the proviral genomes were fully replicative, confluent PKCCs (10–12 days of culture) were co-cultured for 24 h with CEMx174 cells; these cells are highly susceptible to various strains of SIV (Roos *et al.* 2000). The CEMx174 cells associated with the adherent PKCCs and had to be detached by gentle tapping.

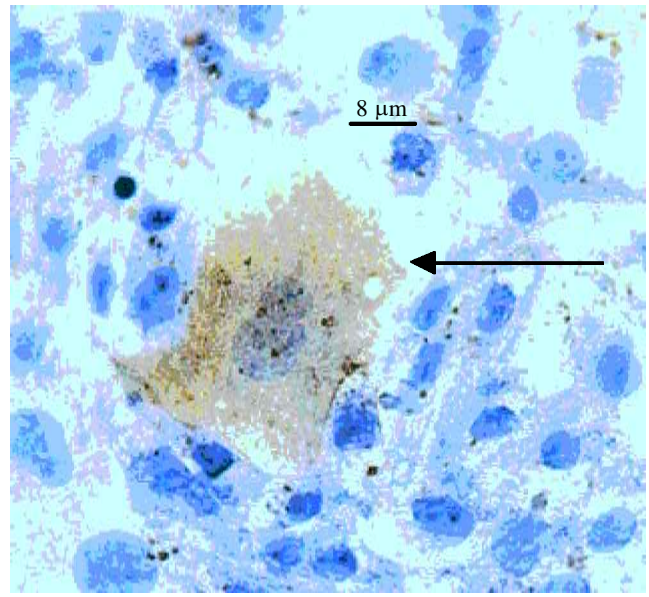

Figure 1. Immunodetection of HAM56-positive cells in a PKCC monolayer. A labelled cell (brown) with macrophage morphology is shown by the arrow.

Harvested CEMx174 were resuspended in RPMI medium supplemented with 10% foetal calf serum and passaged twice weekly for one month. At each passage, the cultures were examined for the presence of giant multinucleated cells as an indicator of SIV replication. The cells co-cultivated with PKCC from monkey Mmu 29697 did not score positive for syncytia. Syncytia were observed at the sixth passage in CEMx174, which had previously been co-cultured with PKCC from monkey Mmu 28824. Immunocapture enzyme-linked immunosorbent assay for viral p27 was positive at passage 6 for the CEMx174 cells exposed to PKCC from Mmu 28824, but remained negative for cultures exposed to PKCC from monkey Mmu 29697.

(c) *Macrophages in kidney cell cultures*

The induction of renal pathology in infected macaques by SIV or SHIV is associated with viral tropism for cells of the macrophage lineage (Stephens *et al.* 1998; Liu *et al.* 1999). We examined our PKCCs for macrophages by immunostaining with mouse anti-human macrophage monoclonal antibody (HAM56; DAKO Corporation, Carpentaria, CA, USA). Cells grown on plastic Lab-Tek[1]

slides were fixed in formalin and incubated with a 1:100 dilution of HAM56. Endogenous peroxidase activity was inhibited by 10 min pre-incubation with 3% hydrogen peroxide, then antibody uptake was determined using the Catalyzed Signal Amplification for Mouse Primary Antibodies System (ref. K1500; DAKO). Cells were counterstained with Mayer's haematoxylin and examined under the light microscope. Cultures contained a small number of positively staining cells with a macrophage-like morphology (figure 1).

4. CONCLUSION

Rhesus macaques are not natural hosts for SIV, and, unlike natural host species, suffer symptomatic infection. Here, we show that the pathogenic SIVmac251 is not expressed in a PKCC from a heavily infected macaque. However, proviral genomes can persist in a potentially replicative state for over 12 days in such a culture. We established PKCCs from kidney specimens taken at necropsy from two rhesus macaques that had been experimentally infected with SIVmac251. The two subjects had a 15-fold difference in plasma viraemia at the time of necropsy. A larger number of copies of proviral DNA were present in fresh kidney and spleen, as well as PKCC, from the animal Mmu 28824 with the higher plasma viral load. Macaque Mmu 28824, however, had a greater number of circulating $CD4^+$ T lymphocytes than its less-severely infected companion, Mmu 29697. As previously reported by Garrett *et al*. (1993), we were unable to detect viral Gag antigens in the supernatant culture medium from PKCCs from either animal. Upon 24 h co-culture with highly susceptible lymphoid cells, virus was transferred only from the PKCC obtained from macaque Mmu 28824. This virus produced a cytopathic effect only after six further passages of indicator cells. The detection of Gag p27 antigen confirms that the cytopathic effect was SIV-induced. The virus probably derives from cells of the macrophage lineage, which we showed to be present in the PKCC and which may originate from either tissue or blood of the donor animal. The absence of viral p27 in the supernatants of PKCC using a sensitive assay suggests that the transfer of virus to the susceptible lymphoid indicator cells on co-culture may have occurred through direct cell contact, possibly facilitated by the allogeneic nature of the CEMx174 cells for the PKCC. This observation may be significant for the attempts to use animal organs for xenografts.

We thank Lou Adamson and Virginia Doucett for help in experimental design and execution, Joanne Higgins, Jin Ling Li and Judy Walls for excellent technical assistance, Dr Linda Antipa-Hirst and the staff at the CRPRC for animal maintenance and procedures and Dr Richard Reyes for generously providing the HAM56 antibody. We especially thank Tim Greenland for helpful discussions during manuscript preparation.

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